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Application Note

Rapid Counting of Somatic Cells in Dairy Milk Using the Scepter™ 2.0 Cell Counter

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Introduction

Mastitis is an inflammatory change in the mammary gland most frequently characterized by pathological changes in mammary tissue. This potentially fatal infection is the most common and costly disease facing the US dairy industry[1]. Milk from cows with mastitis exhibits increased somatic cell counts (SCC) due to the release of white blood cells into the gland to combat infection. Somatic cell counts (SCC) typically range from 2 to >7.5 x10^5 cells/mL, depending on the presence or absence and the degree of disease (Table 1). Samples displaying SCC ≤ 200,000 cells/mL are considered healthy, values ≥ 300,000 cells/mL are indicative of early stages of infection (subclinical mastitis), and counts ≥ 750,000 cells/mL denote full-blown mastitis.

Due to a lack of physical indications, subclinical mastitis cases present the highest cost burden to the dairy industry. To combat this critical issue, research efforts have been focused not only on therapeutic interventions, but also on the design of facile, rapid detection methods to determine elevated SCC counts prior to physical symptoms. Milk is an emulsion of fat globules in a water-based fluid. Milk fat globules possess a number of attributes that severely hinder accurate somatic cell counting, including:

- Significantly more abundant than cells. Depending on the sample, this difference can be on the order of 5,000x.
- Stick to cells, forming aggregates that must be dispersed prior to size-based detection.
- Diameter range: 0.1-20 µm; globule size overlaps with the average somatic cell diameter (8.5-10 µm)
- Content varies significantly, both cow-to-cow and day-to-day.

Bovine health state	Typical SCC ranges
Normal	≤2 x 10^5 cells/mL
Subclinical mastitis	>3 x 10^5 cells/mL
Acute clinical mastitis	≥7.5 x 10^5 cells/mL

Table 1.

Mastitis stage is characterized by typical somatic cell counts (SCC).

Most current methodologies for measuring SCC in milk involve the use of cell-specific fluorescent dyes to analyze samples via flow cytometry and microscopy. The Scepter™ cell counter provides a rapid, reliable alternative to traditional SCC analysis. This device combines the ease of automated instrumentation and the accuracy of impedance-based particle detection in an affordable, handheld format. The precision-made Scepter™ sensor enables the use of the Coulter principle to discriminate cell diameter and volume at submicron and sub-picoliter resolution, respectively.

While the Scepter™ cell counter serves as a reliable alternative for somatic cell counting, excess fat globules must be removed from milk samples to eliminate fat globule interference in SCC. Herein, we detail the spin-wash procedure for milk sample processing and confirm the robustness of the values obtained with results from internal and external flow cytometry testing.

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Materials and Methods

Samples - Fresh milk samples (40-50 mL) were routinely acquired from a local dairy farm (Dunajski Dairy - Peabody, MA). Samples were stored at 4°C and used within 48 hours of collection. Prolonged storage resulted in appreciable changes to both fat and cell content.

Protocol for dairy milk sample preparation

After standing for 12 to 24 hours, fresh milk separates into a high-fat "cream" layer and low-fat milk layer, due to the lower density of the fat globules in comparison to water. Separation may be accelerated by centrifugation via the spin-wash protocol.

The protocol below describes one spin-wash cycle.

1. Allow milk and phosphate-buffered saline (PBS) to come to room temperature.
2. Add 500 µL PBS to 500 µL milk in a 1.5 mL microfuge tube.
3. Mix quickly by vortexing.
4. Centrifuge at 1,000xg for 2 minutes in a tabletop centrifuge
5. Remove upper cream layer using a cotton swab and pour off remaining skim layer.

   Note: The cream layer will stick to the sides of the tube. Remove as much as possible by gently swirling the cotton swab.
6. Add 1 mL PBS to the tube. Do not re-suspend pellet.
7. Repeat steps 4-6 twice.

   Note: Certain samples may require additional spin cycles to achieve sufficient fat removal for cell detection.
8. After final wash, resuspend the cell pellet in 500 µL PBS by gently pipeting up and down.
9. Acquire cell concentration and cell size data using a Scepter™ cell counter equipped with a 40 µm aperture sensor.
10. The 40 µm sensor has an upper detection limit of 1.5 x 10^6 cells/mL. Some samples may therefore need to be further diluted for accurate counting.

Somatic cell counting using the Guava® easyCyte flow cytometer

Aliquots of each purified sample were mixed with ViaCount® reagent (MilliporeSigma), which contains a cell-permeant nuclear dye that preferentially labels nucleated cells.

Stained samples were analyzed on the Guava® easyCyte flow cytometer (MilliporeSigma). 10,000 total events were acquired for each sample.

External SCC testing

For each sample, SCCs were verified by Agri-Mark Dairy Cooperative (Lawrence, MA). Samples were tested using a Fossomatic™ cell counter (Foss, Denmark), a fluorescent-based flow cytometry platform.

Percent recovery studies

Jurkat cells were labeled using CellTracker Green CMFDA (5-chloromethylfluorescein diacetate, Life Technologies). Dye was re-suspended in DMSO to 10 mM. Cells were washed twice with PBS, then labeled by incubating 1 x10^6/mL in fresh culture media at 37°C for 30 minutes with 1:1,000 dilution of dye. Labeled Jurkat cells were added to milk samples at known concentrations. Following a short equilibration time, milk samples were processed and analyzed using the Guava® easyCyte flow cytometer.

Results

Effectiveness of spin-wash method

To determine the effectiveness of fat globule removal, a control and two samples were processed using the following conditions: (1) 1:1 dilution in PBS, (2) one spin-wash, and (3) three spin-washes. Processed samples were analyzed with the Scepter™ cell counter using 40 µm sensors. The results presented in Figure 1 are from a sample with high SCC. The top row displays diameter histogram plots acquired on the Scepter™ cell counter. Initial attempts at analysis of diluted whole milk proved unsuccessful; over half of the samples resulted in sensor failure due to either "Lost Start" errors (caused by air bubbles or insufficiently submerged sensors), or by blocked apertures. Samples that could be read displayed concentration values that greatly overestimated true SCC values due to fat globule interference. For samples treated with three spin-wash cycles, samples measured with the 40 µm sensor showed a clear, distinguishable peak corresponding to the somatic cell fraction. While three spin-wash cycles was sufficient to permit visualization of somatic cells in most samples, additional wash steps were required for samples with extremely high fat content.

Somatic cell detection was confirmed through fluorescent labeling using ViaCount® reagent and the Guava® easyCyte cytometer. The middle row of histograms shows a distinct peak of fluorescently labeled cells (red), even in the unwashed fraction. As 10,000 total events were collected for each sample, the change in relative amplitude of the fat (green) and cell (red) peaks confirms the removal of fat globules with increased wash cycles. In the bottom row, Forward Scatter (FSC) vs. Side Scatter (SSC) dot plots revealed that successive washes released cells (black) from fat aggregates while decreasing the relative number and size of the fat globules (red).

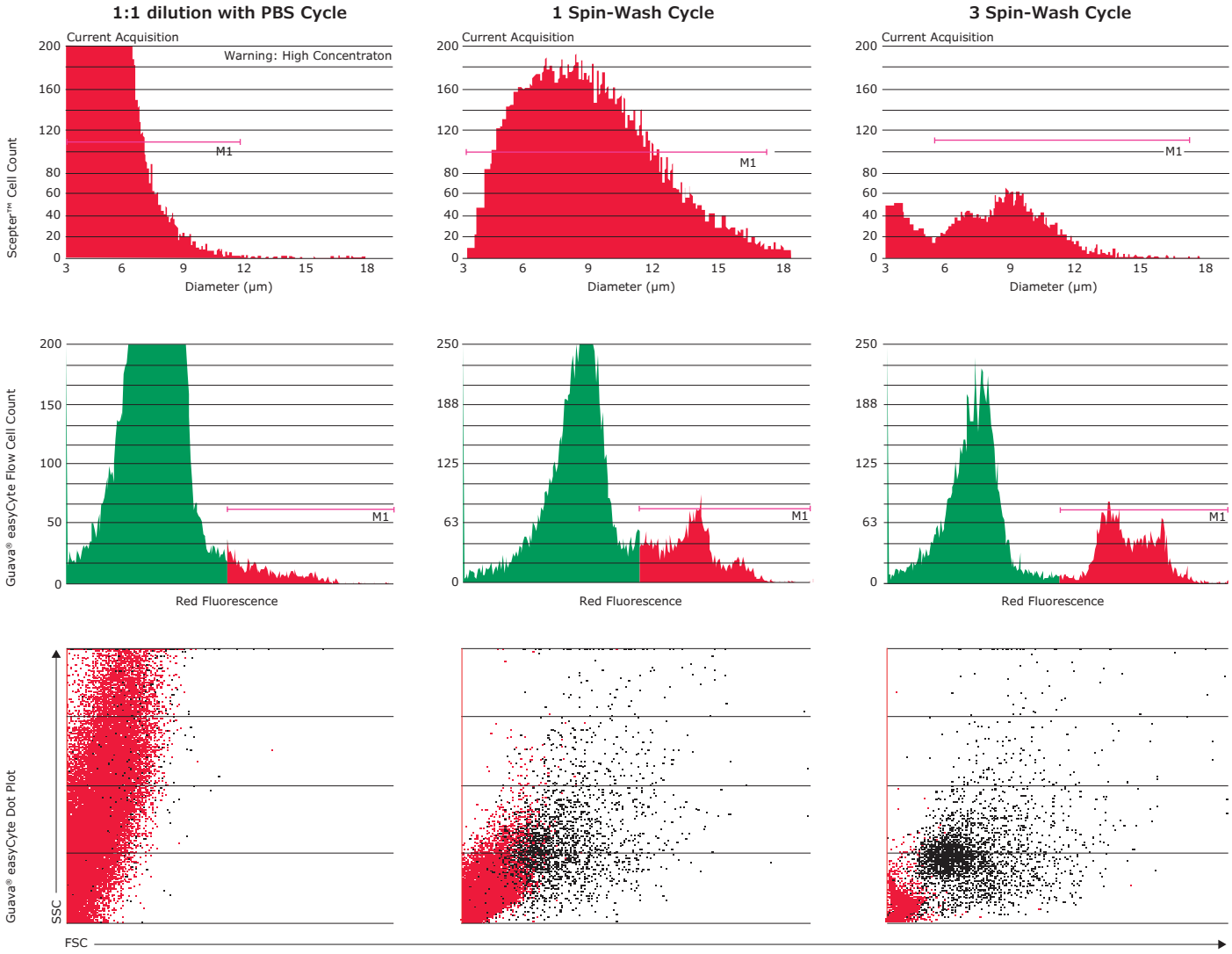

Figure 1.

Spin-wash treatment effectively removes fat globules from dairy milk samples. For Scepter™ counts, events were gated to separate a population of smaller events (green-fat globules) and larger, cellular events (red). For Guava® data, events were gated to separate smaller (low FSC) fat globules (red dots) from larger (higher FSC) somatic cells.

Determining performance range for spin-wash method

Somatic cell concentration can vary greatly depending on diet, temporal proximity to pregnancy, and animal health status. To test the performance range of the spin-wash method, we assessed a number of samples from healthy cows and those presenting with mastitis symtoms. The spin-wash method was successful at enriching for the somatic cell fraction across a wide range of somatic cell counts. Representative examples of low (healthy), medium (subclinical mastitis), and high SCC (acute mastitis) milk samples are displayed in Figure 2. For Scepter™-derived histograms, a distinct peak was seen for all three sample types. In each case, the mean diameter of this peak was consistent with the reported size range for bovine somatic cells. Cell counts were confirmed by flow cytometry. Results presented in the dot plots showed a significant increase in the frequency of detected cells (black) in high SCC samples, corroborating increased peak intensity seen in Scepter™ histograms for high SCC samples.

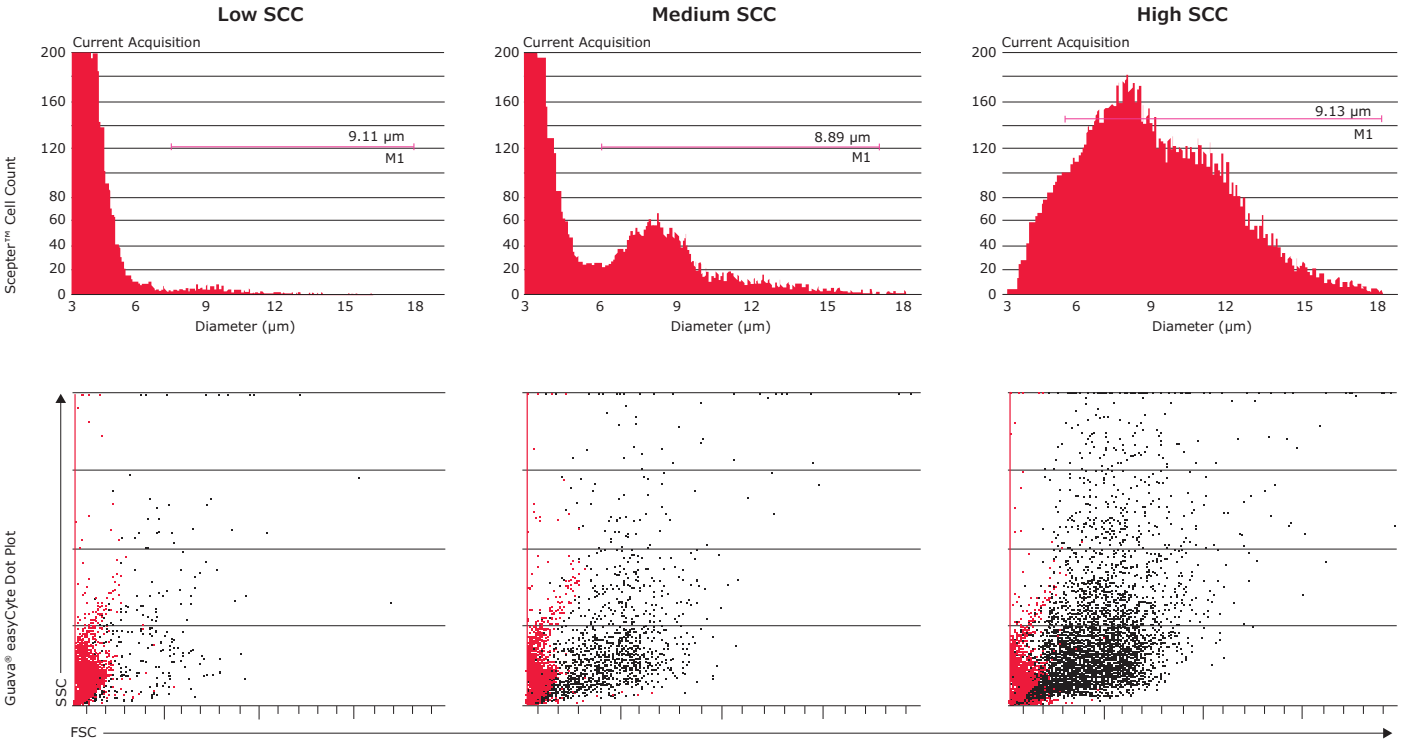

Figure 2.

Scepter™ cell counting and Guava® easyCyte flow cytometry provide interpretable SCC data for dairy milk samples containing low, medium, and high numbers of somatic cells. For the Guava® data, events were gated to separate smaller (low FSC) fat globules (red dots) from larger (higher FSC) somatic cells.

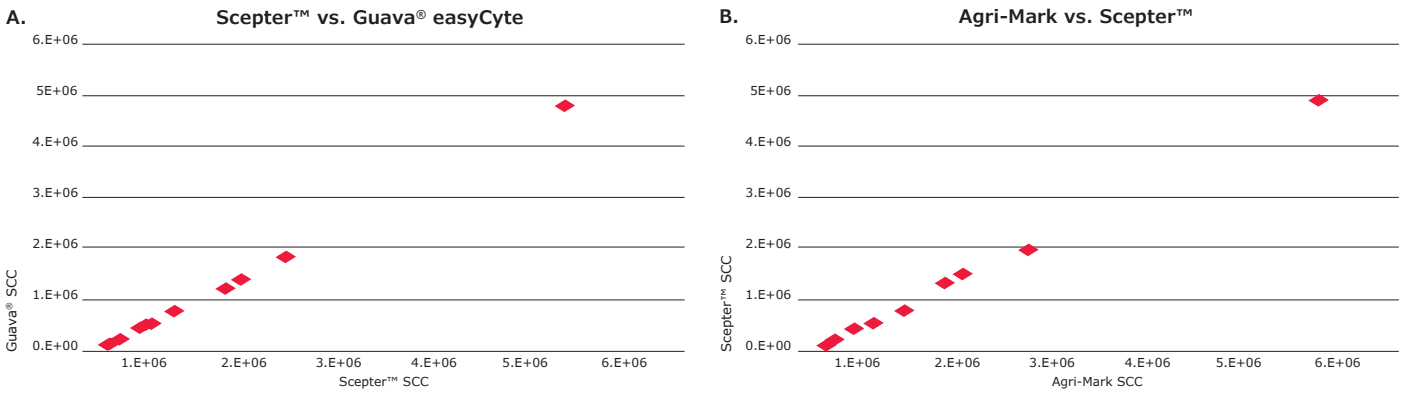

Figure 3.

High correlation of SCC data between three cell counting platforms number of fluorescently labeled cells following ViaCount staining. Agri-Mark is an external SCC testing facility serving the dairy industry.

Twelve samples were processed and analyzed (Figure 3). Overall, the Scepter™-derived SCC showed good agreement with values acquired using the Guava® easyCyte flow cytometer (Figure 3A). However, in nearly all cases, Scepter™ cell counts were slightly higher than flow cytometry values; this difference may be due to greater distinction of cells from fat afforded by fluorescent labeling for flow cytometry. The plotted values represent the mean of three replicates; both platforms showed high reproducibility in sampling results with average percent coefficient of variation (%CV) of 4.5 (Scepter™ cell counter) and 5.0 (Guava® easyCyte flow cytometer). Although slightly lower in all cases, results from both systems were also consistent with those determined externally by Agri-Mark (Figure 3B) on whole milk. This result implies there may be appreciable cell loss attributable to the washing protocol.

Quantifying cell loss with the spin-wash method

To quantify possible cell loss, fluorescently labeled Jurkat cells were added to milk samples at known concentrations. Samples were then subjected to multiple rounds of spin-wash cycles and analyzed by flow cytometry to determine the degree of cell loss (Table 2). While cell loss was slightly increased with lower labeled cell concentrations, cell loss did not exceed 15% in any sample after three rounds of washes. Decreased SCC values for spin-wash processed milk in comparison to SCC values for whole milk can be attributed to minor somatic cell loss during processing, as verified by labeled Jurkat cell counting.

% Cell Recovery			
Initial Jurkat Cell Concentration	**1-Spin**	**2-Spin**	**3-Spin**
50,000	95.6	91.3	85.9
200,000	97.9	92.7	88.7
500,000	97.3	93.7	90.8
1,000,000	97.7	94.1	90.1
3,000,000	98.8	94.8	91.5

Table 2.

Spin-wash sample preparation minimizes cell loss in dairy milk samples.

Conclusion

The Scepter™ cell counter offers the accuracy of impedance-based particle detection in an intuitive and affordable handheld device. The 40 µm aperture sensor provides expanded sensitivity for discrimination of smaller cells and particles. When used in conjunction with the spin-wash protocol, the Scepter™ cell counter enables rapid, precise somatic cell counting, with results comparable to fluorescence-based platforms.

References

1. Viguier, C., Arora, S., Gilmartin, N., Welbeck, K., and O'Kennedy, R. (2009) Mastitis Detection: Current Trends and Future Perspectives. Trends in Biotechnology. 27(8):486-93.

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